That an inverted order is present is again supported by the types of crossover chromatids that could appear if type (1) organization were present but would not appear if type (2) organization had been present in plant 4306 or in plants 4628D-10 and D-11 (table 8). Figure 2 illustrates the types of crossover chromatid that could be anticipated from genic orders (1) and (2) above. Homologous synapsis of the normal chromosome 9 with the distal segment would give phenotypically similar crossover chromatids in the two cases (A, Figure 2). Homologous synapsis of the normal chromosome 9 with the proximal segment, if order (1) were present, could give rise to crossover chromatids having the constitutions shown in B of figure 2. The crossover chromatids that should arise from this association have not appeared. If order (2) were correct, such crossover chromatids would not appear. From this negative evidence, order (2) is again indicated.

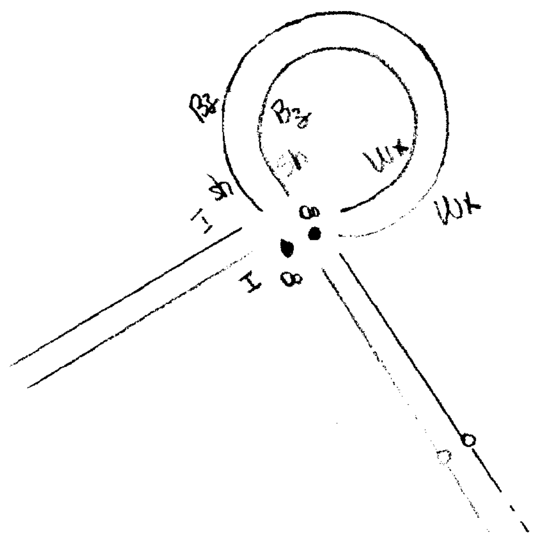
It is possible, now, to reconstruct the events that gave rise to this Duplication chromosome 9 with a transposed Ds locus. Three assumptions regarding these events are required. (1) A Ds mutation occurred at its usual time--late in the development of the sporophytic tissues--in a cell of plant 4108C-1. The chromosome in which this Ds mutation occurred was normal in morphology and carried I Sh Bz Wx and Ds in its standard location. The Ds mutation resulted in breakage of the two sister chromatids at the position of the Ds locus in each chromatid. Evidence that a Ds mutation brings about breaks in sister chromatids at the locus of Ds is well established. This assumption is therefore legitimate. (2) The Ds mutation not only caused breaks to occur at the position of the Ds locus but resulted in the release of a submicroscopic chromatin segment that carries a Ds locus. This released segment carrying Ds has unsaturated broken ends. It could be lost from the chromosome complement if fusion with some other broken ends did not occur. Loss of the Ds locus as a consequence of Ds mutations has been considered in detail elsewhere (see report on c-ml mutations, January, 1949). The manner of this loss may be suggested from cases such as the one being described. (3) At the same time that the events described in (1) and (2) occurred, a spontaneous chromosome break occurred just to the right of the I locus in this chromosome. Both sister chromatids were broken at the same locus. Evidence for frequent spontaneous breaks in maize is good (McClintock, unpublished). This assumption, therefore, is legitimately taken. These three events would give a series of broken ends as shown in A, figure 3. Fusion of broken ends could readily occur to give rise to the configuration shown in B, figure 3. The resulting chromatids are diagrammed in C, figure 3. A Duplication chromosome 9, with an inverted order of genes in the proximal duplicated segment and having a transposed Ds locus just to the right of the I locus is now formed.

On the basis of the depicted mode of origin of the Duplication chromosome 9, it must be assumed that the two Ds loci are daughter Ds loci derived from reduplication of a mother Ds locus. The mutational behavior of the two Ds loci in this chromosome are not alike. More dicentric chromatid-forming mutations occur at Ds^1 than at Ds^2. The states of the two Ds loci definitely differ from one another. This is particularly apparent when these two loci are separated by crossing over and the crossover chromatids isolated. Then, the mutational behavior of Ds^1 may be directly compared with that of Ds^2. Either a change in state occurred in one or both of the Ds loci during one of the events that gave rise to the duplication and the transposition; or a change in state in one or both Ds loci took place subsequent to this event. There is no evidence for any position effect associated with the altered genic associations of the two Ds loci.

Figure 3

A.

Position of chromosome breaks



B.

Fusion of broken ends

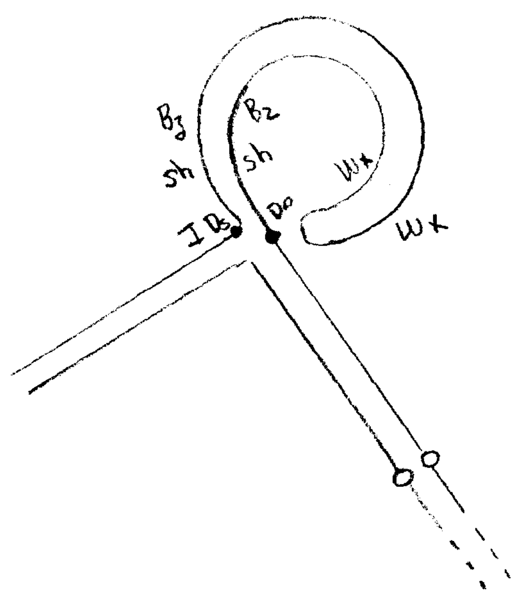
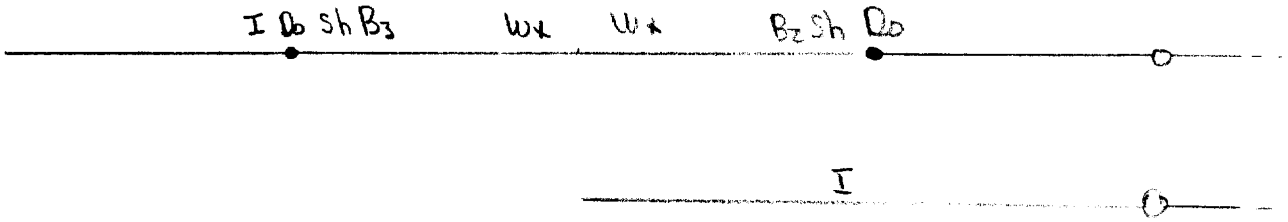


Figure 3 C.

Resulting chromatids



Changes in state of these two separated Ds loci are occurring independently of their positions as a few of the variegated kernels have shown.

Again, it should be emphasized that the transposition of the Ds locus has not introduced any visible alteration in the appearance of the chromosome in the region of the transposition. Neither has its insertion affected the crossing over in the I to Sh region. To refresh this evidence, a summary of this crossing over in the crosses of plants in sub-cultures 4628 F, G and H are given in table 20. These observed crossover percentages are similar to those observed when no Ds locus is present. The inserted segment carrying Ds must be very minute--obviously submicroscopic in size.

The analysis of this case of transposition of Ds has indicated the method for selecting those relatively rare kernels with newly arising transposed Ds loci. In the crosses of I Sh Bz Wx Ds-standard to C sh bz wx ds ac plants, the kernels with aberrant variegation of quite specific types may be selected. It is possible, by this method, to detect those chromosomes having Ds inserted to the left of I, between I and Bz and between Bz and Wx. A number of such kernels have been selected and an analysis of the transposition of the Ds locus is being conducted. Evidence from these other cases should be well advanced by the time the greenhouse crop is collected and certainly by the time the summer crop is harvested.

Table 20

Summary of crossing over between I and Sh in plants 4628F-1, 4628G-1, G-2 and G-3, and plant 4628H-1

$$\frac{\text{Normal chromosome 9} \quad I\ Ds^{1}\ Sh}{\text{Normal chromosome 9} \quad C\ ds\ \ sh}$$

Table reference	Total Number of kernels	Number of cross-over chromatids	Percent Crossing over
Table 9, 4628F-1	564	28	4.9
Table 11-a, 4628b	1461	85	5.5
Table 11-b, 4628b	1243	49	3.9
Table 12, 4628H-1	525	19	3.6
Totals	3793	181	4.7

First rough draft

Appendices to:

Transposition of the Ds locus, April, 1949.

In the previous account the Case I transposition of the Ds locus, April,1949. the origin and behavior of this case of transposition was analysed in considerable detail. Continued examination of this case has resulted in confirmation of the conclusions given in the earlier report. In this supplement, the confirmatory evidence will be given in the form of appendices. The table numbering will follow sequentially from the previous report.

Appendix 1

In the cross given in table 7-c, an Ac ac, C sh bz wx ds female plant was crossed by plant 4628C-9. This latter plant was homozygous for the duplication chromosome 9. Both chromosomes carried I Ds^1 Sh Bz Wx Wx Bz Sh Ds^2. The plant had one Ac locus. A number of kernels appeared on the ear that showed only a few specks of C color (column 1, table 7-c). It could be anticipated that these kernels had 3 Ac loci in their endosperm cells, two contributed by the female parent and one by the male parent. If the Ac loci in both plants were located in allelic positions, the plants arising from these kernels should be Ac Ac. Some of these lightly speckled kernels were selected from the cross of 4462C-11 x 4628C-9 (Table 7-C) and plants grown from them in the summer of 1949 under culture number 4876A. These plants were crossed to: (1) C sh bz wx, ds ac plants (Tables 21-a and 21-b), (2) by C sh bz wx, ds ac plants (Table 22), and (3) to c sh Bz wx, ds ac plants (table 21-c).

In cross (1) above, the types of male gametes, with respect to chromosome 9 morphology and genic constitution, should be the same as those given in the supplement to Table 8. It was hoped, however, that two allelic Ac loci would be present in the tested plants so that a more direct analysis of the chromosome and genic constitutions of the resulting progeny would be available. The four tested plants in Table 21-a had two Ac loci. Unfortunately, however, these two loci did not occupy allelic positions, as the results given in this table indicate. The classes of kernels and their frequencies are those expected if these four tested plants had two non-linked, non-allelic Ac loci. This is shown in the supplement to Table 21-a. It must be concluded, therefore, that the Ac locus in the two parent plants occuppied different positions in the chromosomal complement. In all other respects, however, the results are the same as those given by plants having the same chromosome and genic organizations that were tested in the previous season, Table 8. Therefore, no further description or analysis is required.

In one plant of culture 4876A, the Ac constitution of the main stalk and the tiller differed. This is shown by the frequencies of kernel types obtained when pollen from the main stalk and pollen from the tiller were used in crosses to C sh bz wx, ds ac plants, Table 21-b. The ratios of kernel types obtained when pollen of the main stalk was used indicate that only one Ac locus was present. The pollen from the tiller gave ratios of kernel types that indicate the presence of two non-linked, non-allelic Ac loci. It cannot be decided from these tests whether or not the tiller gained an Ac locus or whether or not the main stalk lost an Ac locus. Either event could account for the observed difference in the two parts of the plant.

Two of the plants entered in Table 21-a were crossed to c sh Bz wx, ds ac plants. The types of kernels appearing on the resulting ears are given in Table 21-c. As the supplement to Table 21-c indicates, the types of kernels and the ratios observed are those expected from the stated constitution of these plants.

In Table 7-c, one aberrant kernel was recorded. This kernel had the phenotype C Sh Bz Wx and was non-variegated. Such a kernel was not expected to appear following the cross given in Table 7-c. A plant was grown from this kernel. It was crossed to a c sh Bz wx, ds ac plant. There were 345 kernels on the resulting ear. 51 were C Sh Wx, non-variegated; 2 were C sh Wx, non-variegated and 292 were C sh wx, non-variegated. When the plant was crossed to a C sh be wx, ds ac plant, the resulting ear showed 27 C Sh Bz Wx non-variegated kernels, 23 C Bz → C bz, Sh, Wx→wx (bz areas wx) kernels, 2 C sh bz Wx non-variegated kernels and 116 C sh bz wx kernels. The plant was likewise self-pollinated. The resulting ear showed 229 C Sh Bz Wx (a number were variegated) one C sh bz Wx and 156 C sh bz wx kernels. If the tested plant had the constitution Duplication C sh bz Wx Wx Bz Sh Ds/C sh bz wx, ac ac, just such ratios following the given cross would be obtained (see Tables 15-b, 15-c and Tables It is suspected that contamination was responsible for the appearance of the aberrant kernel in Table 7-c, or that the pollen grain that gave rise to this kernel had a deficiency in the distal segment that included the I, Ds, Sh, and Bz loci. Only further tests could distinguish between these two possibilities.

In the April 1949 report, no evidence was presented for the transmission of the duplication chromosome through the egg parent. Plants heterozygous for a normal and a duplication chromosome 9 give reduced transmission frequencies of the duplication chromosome through the pollen. The ratios of ~~the~~ C Sh Bz Wx kernels to ~~the~~ C sh bz Wx and C sh bz wx kernels in the crosses of C sh bz wx by Duplication C sh bz Wx Wx Bz Sh Ds/normal chromosome 9 with C sh bz wx indicate the transmission frequencies through the pollen of the duplication chromosome 9. Compilation of the data from Tables give a ratio of Duplication chromosomes 9 to normal chromosomes 9. Such a reduction in transmission of the duplicated chromosome is likewise evident when plants of the constitution I Ds^1 Sh Bz Wx Wx Bz Sh Ds^2/ C sh bz wx are crossed to C sh bz wx. Because the crossing-over between the distal Wx locus and the proximal segment is low, the frequency of the Wx to the wx class gives the approximate frequencies of transmission of the duplication and the normal chromosome. The combined data from Tables 8, 21-a, 21-b and 21-c show 1694 Wx to 3331 wx kernels. In the reciprocal cross, no such reduction in transmission of the duplication chromosome is expected. Four of the plants entered in Table 21 were crossed reciprocally. The results of these reciprocal crosses are given in Table 22. Because of the presence of 4 Ac loci in the endosperms of many of the kernels, it was not possible to make an accurate classification for the presence or absence of Ac in all the kernels. In the table a significant reduction in the Wx class is evident. This is probably not related to a reduction in transmission of the duplication chromosome. Rather, it is related to Ds mutations occurring before meiosis that produced non-female transmissible deficiencies.

Data given in Tables 21 and 22 show that crossing-over between the normal chromosome and the distal duplicated segment in the Duplication chromosome 9 is relatively little affected by the presence of the duplication. This would suggest that synapsis is not seriously disturbed by the presence of the duplication; otherwise, a considerable reduction in crossimg-over between I and Wx would be evident.

Appendix 2

In the summer of 1948, plant 4628K-1 (Tables 14-a and 14-b) having the constitution Dup. C sh bz Wx Wx Bz Sh Ds/C sh bz wx, Ac/Ac, was crossed by plant 4628L-1 (Tables 16-a and 16-b) with the constitution Dup. C Sh Bz Wx Wx Bz Sh Ds/C sh bz wx, Ac Ac. It was hoped that some of the plants arising from the C Sh Bz Wx class of kernels on the resulting ear would be homozygous for the duplication and for Ac. The ear was small and only a few kernels of the desired type were available for testing. Six of these kernels were planted in the summer of 1949 under culture number 4898 but only 4 of them germinated. The constitution of three of these plants were exactly like the female parent plant (4628K-1, Table 14) as the results of reciprocal crosses with C sh bz wx, ds ac plants have shown, Tables 23, 24, and 25.

One of the plants in culture 4898 (4898-2) had the constitution Dup. C sh bz Wx Wx Bz Sh Ds/C Sh bz wx, Ac Ac. The duplication chromosome must have been contributed by the female parent plant whereas the normal chromosome with C Sh bz wx arose from a crossover in region 1 of the male parent plant (see supplement to Table 16-a). Reciprocal crosses of this plant with C sh bz wx, ds ac plants are given in Tables 26-a and 26-b. The cross of this plant to a c sh Bz wx, ds ac plant gave the kernel types listed in Table 26-c. Crossing-over between Sh and Wx in this plant was very high in the microsporocytes but a much lower frequency of recovered crossover chromatids came from the megasporocytes. Such high rates of crossing over in this segment of chromosome 9 have been encountered when two normal chromosomes have been present. A reduced amount of crossing-over in the megasporocytes as compared with the microsporocytes has likewise been observed in numerous tests.

Appendix 3.

In the crosses of C sh bz wx, ds ac plants by Ac ac plants having the Duplication chromosome 9 with I Ds^1 Sh Bz Wx Wx Bz Sh Ds^2 and a normal chromosome 9 with C sh bz wx, a number of distinctive kernel types appeared (Table 8). The projected constitutions of these kernels are given in the supplement to Table 8. In order to determine whether the projected constitutions were correct, some of the kernels from the cross-over classes were selected from two of the crosses, 4462C-8 x 4628D-11, and 4363-17 x 4628D-11 (Table 8) and grown in the summer of 1949 under culture numbers 4877 and 4878, respectively. Two plants arising from the I$\rightarrow$C Bz$\rightarrow$C bz, Sh$\rightarrow$sh, wx kernels (4877A), four plants arising from the C Bz$\rightarrow$C bz, Sh$\rightarrow$sh, Wx$\rightarrow$wx kernels (4877C) and the plants arising from two C Sh bz wx kernels (4877E) in cross 4462C-8 x 4628D-11 were tested for their chromosome 9 constitutions. In the cross 4363-17 x 4628 D-11 (Table 8), four plants arising from the C Bz-C bz, Sh-sh, Wx-wx kernels (4878D)were tested for their chromosome 9 constitutions.

Table 29 gives the ratios of kernel types obtained when the two plants arising from the I Bz$\rightarrow$C Bz$\rightarrow$C bz, Sh$\rightarrow$sh, wx kernels were crossed by C sh bz wx, ds ac plants. The results are those anticipated from the given constitions shown in the heading to this table. The plants arising from the C Bz-C bz, Sh-sh, Wx-wx kernels should all have the Duplication chromosome 9 but the genic constitution could be of several types, as the supplement to Table 8 and Figure 2 illustrate. Crossing over in regions 1 to 5 would give the following genic constitutions in the duplication chromosome:

Region 1: Dup. C Ds^1 Sh Bz Wx Wx Bz Sh Ds^2

Region 2: Dup. C Sh Bz Wx Wx Bz Sh Ds^2

Region 3: Dup. C sh Bz Wx Wx Bz Sh Ds^2

Region 4: Dup. C sh bz Wx Wx Bz Sh Ds^2

Region 5: Dup. C sh bz wx Wx Bz Sh Ds^2

Because crossing-over in region 4 is the highest, the majority of the C Bz-C bz, Sh-sh, Wx-wx kernels in the cross shown in Table 8 should give plants with the constitution: Dup. C sh bz Wx Wx Bz Sh Ds/ C sh bz wx, Ac ac. Of the 8 tested plants arising from such kernels,

were definitely Dup. C sh bz wx Wx Bz Sh Ds/C sh bz wx, Ac ac (Table 27, a and b), were Dup. C sh bz Wx(or wx)Wx Bz Sh Ds/ C sh bz wx, Ac ac (Table 27-d). The tests of the latter plants were inadequate for determining the presence or absence of the distal Wx locus. One plant, 4878D-1, had the constitution Dup. C sh Bz Wx Wx Bz Sh Ds/ normal chr. 9 C sh bz wx, Ac ac, as the results of the cross of this plant by a C sh bz wx, ds ac plant indicate (Table 28). The constitution of the duplication chromosome in this plant resulted from a crossover in region 3 of the parent plant.

In cross 4462C-8 x 4628D-11, Table 8, the 2 C Sh Bz wx kernels could be expected to appear following a double crossover in regions 2 and 4 in the male parent. A normal chromatid carrying C Sh Bz wx would result from such a double crossover. The plants arising from these two C sh bz wx kernels were crossed by C sh bz wx, ds, Ac ac male plants. The results of this cross, Table 30, indicate the presence of two normal chromosomes 9 in each plant. No evidence of a Ds locus in the C Sh Bz wx chromosome appeared in either case.

Appendix 4.

Table 14 gives the types of kernels appearing when a Duplication C sh bz Wx Wx Bz Sh Ds/ Normal C sh bz wx, Ac Ac plant was crossed to a C sh bz wx, ds ac plant. With regard to genic constitutions of chromosomes 9, four types of gametes could be expected to be produced by the male parent. These are:

Non-crossover chromatids

(1) Dup. C sh bz Wx Wx Bz Sh Ds

(2) Normal C sh bz wx

Crossover Chromatids

(3) Dup. C sh bz wx Wx Bz Sh Ds

(4) Normal C sh bz Wx

The genic constitution of the chromosomes 9 of seven plants arising from the C Bz-C bz, Sh-sh, Wx-wx kernels of Table 14-a were tested. 96 to 98 percent of the plants arising from these kernels should have the constitution Dup. C Sh bz Wx Wx Bz Sh Ds/Normal C sh bz wx, Ac ac. All seven tested plants had this constitution. Six plants were crossed by C sh bz wx, ds ac plants, Tables 31-a. All seven plants were crossed to C sh bz wx, ds ac plants, Table 31-b. One plant was crossed to a c sh Bz wx, ds ac female plant, Table 31-c.

Two kernels in Table 14-a were classified as possible C Sh bz wx kernels. Such phenotypes were not expected in this cross. To determine if Sh was actually present, plants were grown from these two kernels and self-pollinated. The self-pollinated ears showed only C sh bz wx kernels. The Sh classification in Table 14-a is therefore erroneous, as anticipated. These two kernels should be moved to the last column in Table 14-a.

Appendix 5.

In Table 16-b, which gives the types of kernels appearing from the crosses of c sh Bz wx, ds ac female plants by a Dup. C Sh Bz Wx Wx Bz Sh Ds, Ac Ac plant, a single aberrant kernel was observed. It was colorless and showed Sh → sh and Wx → wx variegation This kernel was sown in the summer of 1949 and the plant arising from the kernel was crossed to a C sh bz wx, ds ac plant and a c sh Bz wx, ds ac plant. The types of kernels appearing on the two resulting ears indicated that this plant had the constitution I Sh Bz wx Ds Ac/ c sh Bz wx ds ac. This constitution could not have been produced by plant 4628L-1. The aberrant kernel in Table 16-b, therefore, represents a pollen contamination and should be removed from this table.

Appendix 6.

In the crosses of C sh bz wx, ds ac plants by Dup. C sh bz Wx Wx Bz Sh Ds/Normal C sh bz wx plants, Tables 14-a, 15-a and 15-c, a few C sh bz Wx kernels appeared. These were interpreted to arise from crossovers as indicated in the supplements to these tables. They should have two normal chromosomes 9. Plants from seven such kernels were examined cytologically and all seven showed two morphologically normal chromosomes 9. Two of the plants were self-pollinated, Table 32-a, and two were crossed by C sh bz wx plants, Table 32-b, and one was crossed to C sh bz wx, Table 32-c. The expected ratios for Wx and wx were obtained.

Appendix 7.

In the cross of a c sh Bz wx, ds ac female plant by plant 4628L-2 which had the constitution Dup. C sh bz Wx Wx Bz Sh Ds/normal C sh bz wx, Ac ac, an aberrant kernel appeared. This cross is not given in the April 1949 report but is similar to that recorded in Table 17-b. This exceptional kernel was colorless and showed Sh-sh, Wx-wx variegation. A plant was grown from this kernel and crossed to a C sh bz wx, ds ac plant, Table 33-a, and by a C sh bz wx, ds ac plant, Table 33-b. These tests showed that the plant carried a Duplication chromosome 9 with C sh bz Wx Wx Bz Sh Ds. The phenotypic appearance of the kernel from which this plant arose may have been produced following an early spontaneous break that deleted the C locus and initiated the breakage-fusion-bridge cycle that produced the Sh-sh, Wx-wx variegation.

Appendix 8.

In order to obtain plants having a chromosome 9 with I Ds Sh Bz Wx, Ac ac, I Bz-C Bz-C bz, Sh Wx kernels were selected from the ear of a C Sh bz Wx/c sh bz Wx, ac ac female plant by plant 4628G-2 that was I Ds Sh Bz wx/C sh bz wx, Ac ac. Two plants arising from such kernels were crossed to C sh bz wx, ds ac female plants, Table 34. The supplement to Table 34 indicates the types of kernels that should appear following crossing-over. Crossing-over in region 3 gave the desired constitution: I Ds Sh Bz Wx. In these kernels, the C Bz areas were variegated for C bz and the majority of these latter areas were Wx-wx variegated. This is the expected variegation pattern that should be produced from dicentric formations at the Ds locus, immediately to the right of the I locus, which initiates breakage-fusion-bridge cycles.

In these crosses, there were 6 C Bz-C bz, Sh-sh, wx kernels. Such phenotypes could arise from crossovers in region 1. It is possible, however, to obtain such phenotypes from I Ds Sh Bz wx chromatids if a Ds event or events occurs early enough to eliminate the I locus from all of the aleurone cells. This occurs in a small fraction of kernels when Ds mutations take place early in development. Plants arising from all 6 kernels would need to be tested to determine whether or not crossing-over in region 1 or early loss of I following Ds mutations were responsible for the appearance of the C Bz-C bz, Sh-sh, wx phenotype.

Appendix 9.

The position of Ds in this case of transposition is definitely between I and Sh. That it is close to the I locus has been apparent from various crosses previously described. Calculations of the crossing-over between I and Ds (see page 16) gave a percentage of 0.74. This value was derived from the frequency of the C Bz-C bz kernels in the crosses of C sh bz wx, ds ac female x I Ds Sh Bz wx/ C sh bz wx, Ac ac male (Table 11-a).

Tests for the presence of Ds in the C Bz chromosome of plants arising from C Bz- C bz kernels of tables 11a and 12-b, and the C-c kernels of table 11-b and 13-c, were conducted by growing plants from these kernels and testing for the presence of Ds. Twelve kernels were selected from variegated kernels, some from the crosses entered in Tables 11-a, 12-b and 13-c, and some from crosses not recorded in the April 1949 report. Two kernels that were doubtful variegates were likewise selected and the plants grown from them tested for Ds. The various selections are given in the accompanying scheme, Scheme 1.

(Insert Scheme 1, page 43)

Information concerning the origin of the selected kernels, the nature of the variegation, the projected constitution of the derived plant, and the culture number of the plant may be seen from the arrangement in this scheme. The first 10 kernels in the scheme were certain variegates. Nine germinated to give plants and all nine plants were tested for Ds. The last two kernels in the scheme were uncertain variegates. The kernel that gave rise to plant 4885 had

Scheme 1

Cross	Table Reference	Phenotype of selected kernel	Projected constitution of plant arising from kernel	1949 Culture Number
C sh bz wx, ds ac ♀ x I Ds Sh Bz wx / C ds sh bz wx Ac ac ♂				
4363-14 x 4628G-2	11-a	C Bz-C bz, Sh-sh, wx	C Ds Sh Bz wx / C ds sh bz wx	4884
4462C-12 x 4628G-3	Not in April 1949 report	" " "		4887B
C sh bz wx / c Sh bz Wx ♀ x I Ds Sh Bz wx / C ds sh bz wx Ac ac ♂	"	C Bz-C bz, Sh-sh, Wx	C Ds Sh Bz wx / C ds sh bz Wx	4883B
4358A-2 x 4628G-2		C-c Sh Wx	C Ds Sh Bz wx / c ds Sh bz Wx	4883C
Norm. C sh bz wx / Re. c Sh Bz wx ♀ x I Ds Sh Bz wx / C ds sh bz wx Ac ac ♂		C Bz-C bz, Sh-sh, wx	C Ds Sh Bz wx / C ds sh bz wx	4890C-1, 4890C-2
4365-3 x 4628H-1	12-b	C-c, Sh, wx	Norm. C Ds Sh Bz wx / Re. c ds Sh Bz wx	4890D-1, 4890D-2
c sh Bz wx, ds ac ♀ x I Ds Sh Bz wx / C ds sh bz wx Ac ac ♂ 4347-22 x 4628G-2	11-b	C-c, Sh-sh, wx	C Ds Sh Bz wx / c ds sh Bz wx	4886 (no germination)
Re. c sh Bz Wx ♀ x I Ds Sh Bz wx / C ds sh bz wx Ac ac ♂ 4353-2 x 4628G-1	11-b	C-c, Sh-sh, Wx	Norm. C Ds Sh Bz wx / Re. c ds sh Bz Wx	4882
C sh bz wx, ds ac ♀ x I Ds Sh Bz wx / C ds sh bz wx Ac ac ♂ 4684-3 x 4628G-3	11-a	One area only of C Bz-C bz, Sh-sh; wx	Presence of Ds in C Sh Bz wx chromosome uncertain	4885
Re. c sh Bz Wx ds / Norm. C Sh Bz wx Ds ♀ x I Ds sh bz wx / C ds sh bz wx Ac ac ♂ 4380A-8 x 4628I-2 AcAc	13-c	Possible C-c kernel but not certain	Presence of Ds in C sh bz wx chromosome uncertain.	4891

only an area of C Bz - C bz variegation. Tests for Ds were negative. The variegated area in the kernel from which this plant arose may have come from a spontaneous breakage in the chromosome 9 carrying C Sh Bz wx loci in one cell mid-way in development of the kernel. The kernel that gave rise to plant 4891 was not certainly variegated. A few specks that could have been interpreted to be c in phenotype appeared in this kernel. These c specks were suspected to be the result of poor color development. It was thought wise to test for Ds in the plant arising from this kernel. No Ds was present in plant 4891.

The nine plants arising from the ten certain variegated kernels were tested by crossing to (1) C sh bz wx, ds ac, (2) c sh Bz wx, ds ac, and to (3) Rearranged chromosome 9 c sh Wx, ds ac female plants, Tables 35 to 41.

The types of kernels appearing on the ears when plants 4883B and C were crossed to C sh bz wx, ds ac plants are given in Table 35. The presence of Ds to the left of Bz is indicated in the supplement to Table 35. The variegated kernels may be used to obtain the crossover frequencies between Ds and Bz. This gives a frequency of 5.8 percent which is the amount expected if Ds were immediately to the right of the C locus. Plant 4883B was crossed to c sh Bz wx, ds ac female plants giving the kernel types shown in Table 36. A high percent of crossing-over between Ds and wx is to be expected, giving a chromosome with C Ds Sh Wx. When Ac is present, such a chromosome will give C-c variegation in the indicated cross. The c areas should be Wx-wx variegated because of the formation of dicentric chromatids just to the right of the C locus. The presence of Wx-wx variegation was evident in all of the C-c kernels that carried Wx. The presence of Ds to the right of the C locus was indicated by the absence of twin areas with deep color in one area and colorless in its twin area.

Plant 4883C when crossed as a pollen parent to c sh Bz wx, ds ac plants, gave the kernel phenotypes entered in Table 37. Crossing-over between Ds and wx, based on the C-c kernels, was 29.4 percent. The data entered in Tables 35, 36, and 37 are consistent in placing the Ds locus immediately to the right of the C locus. It may be concluded, therefore, that the kernel giving rise to plant 4883B and plant 4883C received a chromosome 9 that was derived from a crossover between I and Ds that had occurred in the I Ds/C ds pollen parent plant (Table 11-a).

The projected constitution of plants 4884, 4887B, 4890C-1 and 4890C-2 was C Ds Sh Bz wx/C sh bz wx, Ac ac. These plants were crossed to plants that were c sh Bz wx, ds ac in constitution. Plants 4884B, 4887B, and 4890C-1 had the expected genic constitution as shown in Table 38. Plant 4890C-2, however, had the constitution I Sh Bz wx/C sh bz wx. No Ds locus was evident. Heterofertilization could account for the discrepancy in kernel and plant phenotypes but the absence of Ds events in the I Sh chromosome of the plant arising from this kernel suggests that a Ds event occurred in the division of the sperm that eliminated Ds from one chromatid (sperm fusing with egg) and initiated the breakage-fusion-bridge cycle in the sister chromatid (sperm entering the endosperm nucleus). The data from the three plants, entered in Table 38, allow the position of Ds in these plants to be calculated. Only the variegated kernels may be used to calculate the crossover percentage between Ds and Sh. The value is 4.5 percent. This is the value expected if Ds is very close to the C locus. It is concluded that these three plants received a chromosome 9 derived from a crossover between I and Ds that placed Ds close to the C locus.

Plants 4890D-1 and D-2 were given the constitutions projected in Scheme 1 (page 43): Normal chromosome 9 with C Ds Sh Bz wx/ Rearranged chromosome 9 with c sh Bz wx, Ac ac. The projection for plant 4882 was normal chromosome 9 with C Ds Sh Bz wx/rearranged chromosome 9 with c Sh Bz wx, Ac ac. If each of these three plants were crossed to plants homozygous for c and sh and had no Ac, C to c variegated kernels should appear on the resulting ears. Because of the presence of the rearranged chromosome 9 in these three plants, the position of Ds in the homologous normal chromosome 9 could not be determined by crossover techniques. The presence of a Ds locus in half of the C carrying kernels (those that have Ac) is evident, however, in the crosses of these plants to plants that were homozygous c sh Wx, ds ac, Table 39. Plants 4882 and 4890D-1 were each crossed to a plant homozygous for c sh wx, ds ac. The results of this test are those expecte, Tables 40 and 41.

None of the data in Tables 35 to 41 gives evidence that allows a determination of the closeness of the Ds locus to the C locus. Tables 9, 11-a, 11-b, and 12-a, however, do show that this transposed Ds is located very close to the I locus. From these tables, the estimated crossing-over between I and Ds is approximately 0.5. The data in Table 34 project [illegible] of approximately 2 percent. This value is much higher than the calculated values from the data in any one of the other mentioned tables. However, several of the six kernels showing a C Bz → C bz phenotype (projected crossovers) in Table 34 may not reflect crossovers ~~may have lost~~ the I locus may have been lost as the consequence of a Ds event that removed the I locus from one or both sperm nuclei. It will be necessary to test the constitutions of the chromosomes 9 in plants arising from each of these kernels in order to determine the reason for the phenotypic expression of each. The combined data indicate, however, that Ds lies to the right of I (proximal) and very close to it.

Table 21-a

C sh bz wx, ds ac ♀ x Dup. I Ds[1] Sh Bz Wx Wx Bz Sh Ds[2] Ac ac, Ac ac ♂
Norm. C sh bz wx

Kernel type	4805B-19 x 4876A-1	4805B-25 x 4876A-1	4807-1 x 4876A-3	4807-8 x 4876A-3	4803-23 x 4876A-4	4803-27 x 4876A-4	4804-10 x 4876A-5	Totals
I Sh Wx	23	37	7	7	33	22	22	151
I Bz - C Bz - C bz, Sh, Wx-wx	111	102	23	28	50	34	82	430
I Sh wx	10	15	7	6	15	17	28	98
I Bz - C Bz - C bz, Sh wx	21	21	16	21	30	26	60	195
I bz - C bz, Sh wx	4	8	4	1	2	2	1	22
I sh wx not obv. var.	4	4	4	4?	6	3	4	29
I bz - C bz, sh wx	5	5	4	0	9	7	3	33
C Sh Bz Ws not obv. var.	15	13	4	7	20	9	13	81
C Bz - C bz, Sh, Wx-wx	26	37	4	14	17	15	19	132
C sh bz wx	201	149	60	76	158	129	254	1027
Odds	1 C Sh Bz wx	0	0	1 C Sh Bz wx	1 C Sh Bz wx (var.?)	1 C Sh Bz wx	2 C sh bz Wx	6
Totals	421	391	133	165	341	265	488	2204

796 Wx : 1408 wy

Supplement to table 21-a

1 2 3 4 5

I Ds Sh Bz Wx Wx Bz Sh Ds[2]

C ds Sh bz wx

Non-crossovers:

Dup. I Ds Sh Bz Wx Wx Bz Sh Ds
- 3 Ac = I - C Bz - C bz Sh Wx-wx = 430 (with reg. 4 + doubles)
- 1 ac = I Sh Wx " " " = 151

Norm. C ds sh bz wx Ac & ac = C sh bz wx = 1027

Region 1

Norm. I ds sh bz wx Ac & ac = I sh wx

Dup. C Ds Sh Bz Wx Wx Bz Sh Ds
- 3 Ac = C Bz - C bz Sh Wx-wx (bz areas Wx-wx or wx)
- 1 ac = C Sh Bz Wx

Region 2

Norm. I Ds sh bz wx
- 3 Ac = I bz - C bz sh wx = 33
- 1 ac = I sh wx

Dup. C ds Sh Bz Wx Wx Bz Sh Ds[2]
- 3 Ac = C Bz - C bz Wx Wx-wx (bz areas wx)
- 1 ac = C Sh Bz Wx

Region 3

Norm. I Ds Sh bz wx
- 3 Ac = I bz - C bz Sh wx = 22
- 1 ac = I Sh wx

Dup. C ds sh Bz Wx Wx Bz Sh Ds
- 3 Ac = C Bz - C bz Sh Wx-wx (bz areas wx)
- 1 ac = C Sh Bz Wx

Region 4

Norm. I Ds Sh Bz wx
- 3 Ac = I Bz - C Bz - C bz Sh wx = 195
- 1 ac = I Sh wx

Dup. C ds sh bz Wx Wx Sh Bz Ds
- 3 Ac = C Bz - C bz Sh Wx-wx (bz areas wx)
- 1 ac = C Sh Bz Wx

Supplement to table 21-a (continued)

Region 5

Norm I Ds Sh Bz Wx	3 Ac = I Bz - C Bz Sh Wx-wx
	1 ac = I Sh Wx
Dup. C ds sh bz wx Wx Bz Sh Ds	3 Ac = C Bz - C bz Wh Wx-wx (bz areas wx)
	1 ac = C Sh Bz Wx

Double crossovers

Regions 2 + 3

Norm. C ds Sh Bz wx Ac + ac	= C Sh Bz wx	=	4
Dup. I Ds sh bz Wx Wx Bz Sh Ds	3 Ac = I Bz - C bz Sh Wx-wx		
	1 ac = I Sh Wx		

Regions 4 + 5

Norm. C ds sh bz Wx Ac + ac	= C sh bz Wx	=	2
Dup. I Ds Sh Bz wx Wx Sh Bz Ds	3 Ac = I Bz - C bz Sh Wx-wx		
	1 ac = I Sh Wx		

Supplement to table 21-a (continued)

Normal chromatids

Non-cross-over	=	1027	C sh bz wx	
Regions 1 + 2	=	62	I sh wx	= I to Sh
Regions 3 + 4	=	315	I Sh wx	= Sh to Wx
Regions 2 + 3	=	4	C Sh Bz wx	
Regions 4 + 5	=	2	C sh bz Wx	
Totals		1410		

% cross-overs region I to Sh = 4.6% (see Table 20 in Report, April, 1949 = <u>4.7%</u>)

% " " " Sh to Wx =22.48%

Conclusion:

Presence of Duplication does not interfere with the normal amount of crossing over in the region between I and Wx of the distal segment.

Ratio of 2, 3 and 4 from variegated kernels with I

Region 2 = 33 I bz - C bz sh wx

Region 3 = 22 I bz - C bz Sh wx

Region 4 = 195 I Bz - C Bz - C bz Sh wx

Ratios = 1.5 : 1 : 9